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ARTICLE

Genetic parameters considering traits of importance for cassava biofortification

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Abstract: *Twenty six sweet cassava clones were evaluated in two agricultural years, in randomized block design, with 3 replications and plots with 25 plants. The harvests were at 12 months after planting, in both years. The traits evaluated were root yield (RY), dry matter content (DMC), total carotenoids content (TC), β-carotene content (BC), trans-β-carotene content (TrBC) and total cyanide content (TCy). Genotype was significant (P<0.05) in all traits. Broad-sense heritability estimates at the plot level (h^2_{mg}) ranged from 77% (RY) to 93% (TC and TrBC), while the accuracy ranged from 0.88 (RY) to 0.96 (BC and TrBC). The genetic correlations among TC, BC and TrBC were high (0.96 to 0.99) and significant, and the genetic correlations involving these traits and TCy were negative. These results demonstrate the existence of great genetic variability in characteristics important to cassava biofortification and, consequently, great perspectives in the breeding for this purpose.*

Keywords: *Mixed models, carotenoids, beta-carotene, sweet cassava,* Manihot esculenta

INTRODUCTION

Cassava is a crop whose main product are the starch-rich roots, being one of the main sources of dietary energy in many tropical countries (Ceballos et al. 2015, Ceballos et al. 2020). The pulp color of cassava roots can be white, yellow, orange or cream (Ayetigbo et al. 2018), and there is a close relation between the pulp color and the carotenoid content in this crop (Sánchez et al. 2014). Carotenoids may be precursors of vitamin A (Mezzomo and Ferreira 2016), which when at deficiency levels in humans causes several problems including permanent blindness (Stevens et al. 2015). One of the carotenoids linked to vitamin A activity in humans is β-carotene (Moura et al. 2015).

Biofortification is the process by which the nutritional quality of the main crops is improved through breeding, a feasible and inexpensive means of providing access to better foods to poorer populations (Bouis and Saltzman 2017, Garg et al. 2018). There are several obstacles to cassava breeding, such as the heterozygosity (which hinders the identification of superior individuals), difficulties of some genotypes in flowering, and the low propagation rate (Ceballos et al. 2020). These difficulties require the use of techniques that increase the probability of selecting genetically superior individuals in this crop.

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Variance components and genetic values are essential parameters in breeding programs, and the Restricted Maximum Likelihood/Best Linear Unbiased Prediction (REML/BLUP) is the standard procedure for obtaining such parameters in several species (Resende 2016). The main advantages of REML/BLUP are: i) it allows the comparison of individuals or varieties throughout time (generations, years) or in different locations (locations, blocks); ii) it allows dealing with complex structured data (repeated measurements, different years, places and experimental designs); iii) and it can be applied to unbalanced data and non-orthogonal designs. REML/BLUP enables a more precise prediction of genetic values and estimation of genetic parameters, as heritabilities and genetic correlations (Resende 2007). BLUP maximizes the correlation between true and predicted genotypic values (Piepho et al. 2008). This work aimed to estimate genetic parameters based on traits of importance in the context of cassava biofortification, using the mixed model procedure.

MATERIAL AND METHODS

Characterization of the experimental area

The work was carried out in the experimental field of Embrapa Cassava & Fruits, in Cruz das Almas, state of Bahia, Brazil, located at lat 12° 39' 11'' S, long 39° 7' 19'' W, alt 199 m asl, with a temperature of 24.5 °C, relative humidity of 80% and average annual rainfall of 1200 mm. The soil was classified as *Latossolo Amarelo Distrocoeso Argissólico* (Oxisol), according to Santos et al. (2018).

Soil preparation consisted of plowing followed by two harrowings. Fertilization was performed based on soil analysis, applying phosphorus (60 kg ha^{-1} of P_2O_5) and potassium (40 kg ha^{-1} of K_2O) at planting, and nitrogen (30 kg ha^{-1} of N) at 50 days after planting. Weeding was carried out during the crop cycle, to always keep the experiment clean.

Genotypes and field evaluations

The 26 clones assessed in this study came from an initial group of 224 clones and belong to 12 families of full sibs, resulting from crosses among accessions from the Cassava Active Germplasm Bank at Embrapa Cassava & Fruits (Table 1). These accessions were crossed due to their yellow pulp roots. Crosses and handling of the seeds were done according to Freitas et al. (2018). The 224 clones were assessed for root pulp color (yellow), according to Sánchez et al. (2006), and 26 with more intense yellow were selected. Later, these 26 clones were evaluated in two agricultural years.

Table 1. Genealogy of 26 clones assessed in this study

Progeny	Number of clones	Parents	
		Female	Male
2003 01	2	1667	1721
2003 03	7	1667	1668
2003 05	1	1668	1692
2003 06	1	1668	1721
2003 07	2	1668	1722
2003 14	4	1692	1721
2003 15	1	1692	1668
2003 17	1	1722	1692
2003 18	2	1722	1721
2003 20	1	1721	1668
2003 23	4	456	1722

The design adopted in both years consisted of randomized blocks, with 3 replications and plots of 25 plants spaced 1.0 m x 0.60 m. Both harvests were carried out at 12 months after planting. After harvesting, the roots were separated from the shoot and weighed using a digital scale (Brecknell ElectroSamson 45 kg x 0.01 kg, Fairmont, Minnesota, USA), obtaining the root yield (RY; t ha^{-1}), and five roots separated from each plot were used for the analyses of carotenoids, cyanogenic glycoside and dry matter.

Laboratory analyses

Root preparation

The selected roots were washed, dried, peeled and divided into four parts through two longitudinal cuts. Two opposite quarters were used for carotenoid and dry matter analysis and the other opposite sides for cyanogenic compounds. The samples were grated using a stainless-steel food processor for extraction and homogenized in a vertical mixer to obtain a homogenous mass.

Determination of carotenoids

The quantification of total carotenoids and β-carotene was performed according to Rodriguez-Amaya and Kimura (2004). A portion (5 to 10 g depending on the pulp color) was homogenized for 1 min with 30 mL of acetone with a Polytron homogenizer (Ultra Turrax IKA T18 digital, Staufen, Germany) and then filtered under vacuum (Vacuum Pump Prismatec 121, Itu, Brazil). The carotenoid solution was made up to volume with petroleum ether and the absorbance was taken at 450 nm (Spectrophotometer Thermo Scientific Genesys 10S UV-Vis, Shanghai, China). The total carotenoid content was calculated using the following formula:

$$\text{Total carotenoid content (TC; } \mu g\ g^{-1}) = \frac{A \times \textit{sample volume}\ (mL) \times 10^4}{A^{1\%}_{1cm} \times \textit{sample weight}\ (g)}$$

where A = absorbance, sample volume = volumetric flask (mL), and $A^{1\%}_{1cm}$ = 2592 (β-carotene extinction coefficient in petroleum ether).

Aliquots (5 or 10 mL) of the petroleum ether solution used for quantification of TC were taken for the quantification of total β-carotene (BC; μg g^{-1}) and *trans*-β-carotene (TrBC; μg g^{-1}), with a high-performance liquid chromatography (HPLC Waters Alliance 2695, Milford, USA) equipped with quaternary pump, autosampler, in-line degasser, UV / visible photodiode array detector between 350 and 600 nm and the C30 column (Waters YCM carotenoid S-3, 4.6 x 250 mm, reverse) controlled by Empower software.

The concentration of β-carotene and its isomer *trans* was determined with the following formula: β-carotene (BC) or *trans*-β-carotene (TrBC) (μgg^{-1}) = $\frac{A_x \times C_s\ (\mu g mL^{-1}) \times V(mL)}{A_s \times P(g)}$

where A_x = carotenoid peak area, C_s = standard concentration, A_s = standard area, V = total extract volume, and P = sample weight.

Total cyanide content

This analysis was performed according to Essers (1994). Approximately 60 g of the homogeneous solution was transferred to a *Büchner funnel* coupled to a 500 mL Buchner flask and filtered under vacuum. The extract was analyzed for cyanogenic compounds after appropriate dilution, by hydrolysis with exogenous linamarase prepared in the lab from cassava cortex extraction (Cooke 1979), and after 10 minutes at room temperature the absorbance was recorded at 605 nm on a spectrophotometer (Thermo Scientific Genesys 10S UV-Vis Spectrophotometer, Shanghai, China). The total cyanide content (TCy) is expressed as μg HCN g^{-1} fresh weight.

Dry matter content

Dry matter content (DMC; %) was determined with the oven drying method (Ige et al. 2022). The moisture from the roots (sample of 100 g) was obtained after drying in forced air circulation oven (Dehydrator Pardal PE60, Petrópolis, Brazil) at 60 °C to constant weight. Dry matter content was expressed as the percentage of dry weight relative to fresh weight.

Statistical analysis

Data on root yield (RY, t ha^{-1}), total carotenoids content (TC, μg g^{-1}), β-carotene content (BC, μg g^{-1}), *trans*-β-carotene content (TrBC, μg g^{-1}), total cyanide content (TCy, μg g^{-1}) and dry matter content (DMC, %) were analyzed by mixed models (Henderson 1974) with fixed effects estimation via BLUE (best linear unbiased estimation), prediction of random effects via BLUP (best linear unbiased prediction) and estimation of variance components via REML (restricted maximum likelihood). Statistical analysis was performed using Selegen-REML/BLUP software (Resende 2016), model 54. The following matrix model was used for such analysis, considering each characteristic individually:

$$y = X_r + Z_g + W_i + e$$

where:

y : vector of phenotypic observations at the plot level for each trait evaluated.

r : vector of the fixed effects of replicates added to the overall mean in each year (replicate-year combination).

g : vector of the random effects of clones, $g \sim N(0, I\sigma^2_g)$.

i : vector of the random effects of clones x years interaction, $i \sim N(0, I\sigma^2_i)$;

e : vector of random residuals, $e \sim N(0, I\sigma^2_e)$;

X, Z and W: incidence matrices (0 and 1) of said effects.

σ^2_g, σ^2_i and σ^2_e: genotypic, clones x years and residual variances, respectively.

To test the random effects of the model (clones and clones x years interaction) nested models were built, that is, a full model and a reduced model without the effect to be tested. With the logarithm of the maximum of the residual likelihood function (L) in hand, the deviances (D = -2 log L) for the full and reduced models were calculated. The difference between the deviances of the reduced and full models allows obtaining the likelihood ratio ($LR = D_{reduced} - D_{full}$), which follows a chi-square distribution. Thus, the test of this ratio, known as likelihood ratio test (LRT), is performed using the chi-square statistic with 1 degree of freedom for each effect under test, at the 5% level.

The selection accuracy was estimated by the expression $r_{\hat{g}g} = \sqrt{1 - \overline{PEV}/\sigma^2_g}$, where $\overline{PEV}$ is the variance of the mean prediction error of the genotypic BLUP. Broad-sense heritability, at the plot level, was calculated by the ratio $h^2_g = \frac{\sigma^2_g}{\sigma^2_g + \sigma^2_i + \sigma^2_e}$. Broad-sense heritability, at the level of genotype means, was calculated by the ratio $h^2_{mg} = \frac{\sigma^2_g}{\sigma^2_g + \frac{\sigma^2_i}{r} + \frac{\sigma^2_e}{re}}$, where r and e are the numbers of replicates and environments (years), respectively. The residual and genotypic coefficients of variation were calculated by the ratio between the standard deviation and the corresponding overall phenotypic mean ($CV\% = \frac{\sigma}{\bar{y}}\ 100$).

From the predicted genotypic values, free from the interaction effect, and the observed phenotypic values, the Pearson correlations between each pair of traits were calculated. For this we used the data of all the 26 clones. The significance of genotypic correlations was tested via bootstrap with 1000 resamplings, in order to construct the distribution of correlations for each trait, at a level of 5%, using the bootstrap package of R software (R Core Team 2022).

RESULTS AND DISCUSSION

Deviance analysis

The effect of clones is significant for all traits (Table 2). Consequently, the variance components, as the heritability estimates, are significantly different from zero. The significance ($P<0.05$) of the clones x years interaction in BC is attenuated by the very high and significant genetic correlation (0.99) between BC and TC (Table 4) and the high estimate of h^2_{mg} (93%) of TC (Table 3), which guarantees that the selection on TC will result in increase in BC.

Variance components

According to Vencovsky (1987), an interesting way to evaluate experimental precision is to obtain the ratio between genetic (CV_g) and experimental (CV_e) coefficients of variation ($b = \frac{CV_g}{CV_e}$). The b values are greater than 1 in all the traits (Table 3), showing that the genetic variance is higher than the environmental variance (Vilela et al. 2022), a favorable situation to the selection.

The heritability estimates of RY, DMC and TCy, based on plots (h^2_g), were around 50%, while those related to carotenoids ranged from 72% (TC and BC) to 75% (TrBC). As expected, heritability estimates based on clone means (h^2_{mg}) were higher

Table 2. Quantile of Likelihood Radio Test (LRT) for random effect model

LRT	RY	DMC	TC	BC	TrBC	TCy
Genotypes	12.31**	19.16**	34.64**	30.4**	33.72**	15.23**
Interactions	12.86**	0.18	0.31	4.1*	3.5	5.6*

RY: root yield; DMC: dry matter content; TC: total carotenoids; BC: β-carotene; TrBC: *trans*-β-carotene; TCy: total cyanide

**: means at the 1% level by Chi-square test ($\chi^2_{0.01} = 6.63$); *: means at the 5% level by Chi-square test ($\chi^2_{0.05} = 3.84$).

Table 3. Estimates for residual coefficient of variation (CV_e), genotypic coefficient of variation (CV_g), CV_g/CV_e ratio (b), genotypic variance (σ_g^2), heritability at plot level (h_g^2), heritability at genotypic means level (h_{mg}^2), genotype selection accuracy, and phenotypic means (for the two years), for sweet cassava genotypic evaluation

Estimates	RY	DMC	TC	BC	TrBC	TCy
CV_e (%)	26.95	6.64	11.04	10.91	13.42	27.26
CV_g (%)	35.56	6.79	18.17	19.88	26.08	32.54
b (CV_g/CV_e)	1.32	1.02	1.65	1.82	1.94	1.19
σ_g^{2*}	23.48	4.67	1.84	1.48	1.74	393.16
h_g^2 (%)	51	50	72	72	75	51
h_{mg}^2 (%)	77	85	93	91	93	81
Accuracy	0.88	0.92	0.96	0.96	0.96	0.90
Phenotypic Mean	13.51	31.79	7.49	6.16	5.09	61.35

RY: root yield; DMC: dry matter content; TC: total carotenoids; BC: β-carotene; TrBC: *trans*-β-carotene; TCy: total cyanide

*: significant at 1% level by chi-square test (Table 2)

Table 4. Phenotypic (above the diagonal) and genotypic (under the diagonal) correlations

Correlation	RY	DMC	TC	BC	TrBC	TCy
RY	-	0.07	-0.05	-0.09	-0.13	-0.19
DMC	0.28	-	0.28	0.29	0.20	-0.38
TC	0.19	0.34*	-	0.97	0.95	-0.33
BC	0.21	0.33*	0.99*	-	0.98	-0.33
TrBC	0.11	0.19	0.96*	0.98*	-	-0.26
TCy	-0.34	-0.58*	-0.37*	-0.39*	-0.27	-

RY: root yield; DMC: dry matter content; TC: total carotenoids; BC: β-carotene, TrBC: *trans*-β-carotene; TCy: total cyanide. *: significant at 5% level

than those of h_g^2, ranging from 77% (RY) to 93% (TC and TrBC). This is understandable, since the phenotypic variance is smaller in h_{mg}^2 (Schmidt et al. 2019).

The highest heritabilities of TC, BC and TrBC indicate that selection to increase carotenoid levels in cassava can start in the initial phases of the program. Heritability estimates in literature for TC vary from 60.58% (Parkes et al. 2020) to 99.2% (Nduwumuremyi et al. 2018). These high estimates support the fact that carotenoid content in cassava roots is controlled only by two genes (Chavez et al. 2000).

Accuracy is the correlation between the predicted and the true genetic values of the individuals (Resende 2002). According to Resende and Duarte (2007), accuracy values around 0.60 are average, from 0.70 to 0.89 are moderate and are high if above 0.90. The accuracy estimates ($r_{\hat{g}g}$) obtained in this work (0.88 in RY, 0.92 in DMC, 0.96 in TC, BC and TrBC and 0.90 in TCy; Table 3) are therefore moderate in RY and high in the other traits.

The RY mean of the 26 clones in this work (13.51 t ha^{-1}) is lower than those obtained by Vieira et al. (2018), Parkes et al. (2020), Peprah et al. (2020): (26.86, 16.23 and 23.43 t ha^{-1}, respectively). Regarding DMC, estimates in the literature range from 24.12% to 28.21% (Araújo et al. 2019, Parkes et al. 2020, Peprah et al. 2020), while the value obtained in this study was 31.79% (Table 3). The dry matter content (DMC) is important in sweet cassava because starch, which corresponds to 65-91% of the dry matter of cassava roots (Sánchez et al. 2009), has a great influence on its cooking (Bechoff et al. 2018), and almost all forms of sweet cassava consumption require the cooking of the roots.

Vitamin A deficiency causes serious health problems (Stevens et al. 2015), and biofortification is an inexpensive way to solve it (Bouis and Saltzman 2017). β-carotene is one of the precursors of vitamin A. The means of TC (7.49 μg g^{-1}), BC (6.16 μg g^{-1}) and TrBC (5.09 μg g^{-1}) obtained in this work are similar or slightly higher than those reported by Carvalho et al. (2012) (TC: 6.53 μg g^{-1}, BC: 3.73 μg g^{-1}), Ikeogu et al. (2019) (TC: 4.72 μg g^{-1}, TrBC: 1.58 μg g^{-1}) and Parkes et al. (2020) (TC: 6.53 μg g^{-1}). However, Sánchez et al. (2014) and Jaramillo et al. (2018) report much higher estimates (TC ranging from 11 to 14.3 μg g^{-1}, BC of 10.1 μg g^{-1}), and Ceballos et al. (2013), report results varying from 2.4 to 14.7 μg g^{-1} in TC, while in BC the increase was from 2.3 to 8.6 μg g^{-1}.

The content of cyanogenic glycosides is crucial in cassava since the final product of this metabolic pathway (HCN) is highly toxic (Mosayyebi et al. 2020). The TCy mean in this work was 61.35 μg g^{-1} (Table 3) and the range was from 35.53 to 110.51 μg of HCN g^{-1} (Table 5). Although the internationally established limit for a cassava clone to be considered sweet cassava is 50 μg g^{-1} (Feeley et al. 2012), Lorenzi et al. (1993) observed that 33% of 206 clones consumed as sweet cassava by Brazilian farmers had levels of cyanogenic compounds above 100 μg g^{-1}. Since then, in Brazil the limit to consider a sweet cassava clone suitable for consumption is 100 μg g^{-1}.

Correlations

The phenotypic (above the diagonal) and genotypic (below the diagonal) correlations are in Table 4. All genotypic correlations involving RY were not significant. A similar result was obtained by Silva et al. (2016), regarding the correlation between RY and starch content. Differently, Parkes et al. (2020) observed significant genetic correlations between RY and DMC (0.16) and between RY and TC (-0.29). All phenotypic correlations involving RY (except RY vs DMC) were negative, while among the corresponding genotypic correlations, the only negative was between RY and TCy (r_g=-0.34). Although the TCy vs RY (-0.34) and TCy vs TrBC (-0.27) correlations are not significant, the fact that all phenotypic and genotypic correlations involving TCy are negative is important, since in this trait the goal is to reduce the mean. The high and significant genetic correlations between TC and its fractions (Table 4) demonstrate that it is possible to increase the levels of β-carotene (BC) and *trans*-β-carotene (TrBC) by making selection over TC, which can make breeding for cassava biofortification faster and cheaper. Similarly, Ikeogu et al. (2019) observed a correlation of 0.97 between TC and TrBC.

Table 5. Average genotypic values (μ+g) for the 26 evaluated sweet cassava clones

Clones	RY	DMC	TC	BC	TrBC	TCy
1	12.86	**33.93**	7	5.43	3.66	**40.71**
2	8.4	31.95	7.25	5.96	4.9	**43.47**
3	**19.08***	30.9	6.89	5.78	4.96	51.55
4	15.19	**33.37**	**10.51**	**8.77**	**7.85**	**38.64**
5	12.5	32.96	**10.03**	**8.40**	**7.69**	54.35
6	13.08	30.28	6.67	5.68	4.8	62.25
7	13.66	31.97	5.29	4.15	2.83	53.51
8	**18.3**	27.15	6.1	4.85	3.88	83.94
9	12.82	30.49	8.01	6.59	5.76	70.46
10	**20.05**	**33.94**	8.41	6.97	**5.9**	**35.53**
11	14.11	32.3	**8.57**	6.62	5.56	82.67
12	7.08	31.73	5.5	4.2	3.3	72.16
13	11.26	32.4	6.58	5.24	3.74	54.26
14	8.37	32.62	**9.02**	**7.52**	**6.69**	55.01
15	17.98	32.48	7.45	5.95	4.43	**46.97**
16	**19.45**	**34.51**	8.27	**7.01**	5.55	50.94
17	15.79	32.84	**8.46**	**7.11**	**5.96**	51.94
18	12.56	31.31	7.39	6.12	5.13	71.04
19	14.34	29.03	7.85	6.6	5.84	50.11
20	11.33	32.15	7.47	6.3	5.37	61.04
21	9.57	27.21	7.25	5.89	5.14	110.51
22	9.14	32.15	7.68	6.38	5.44	80.25
23	**22.88**	**35.78**	6.92	5.74	4.35	66.74
24	16.37	30.49	7.02	5.8	4.35	57.92
25	11.64	32.61	7.56	6.44	5.34	47.21
26	6.45	30.66	4.8	3.85	2.9	90.93
Mean	13.63	31.82	7.46	6.13	5.05	60.93

RY: root yield (t ha^{-1}); DMC: dry matter content (%); TC (μg total carotenoids g^{-1} fresh root), BC (μg β-carotene g^{-1} fresh root), TrBC (μg *trans*-β-carotene g^{-1} fresh root) and TCy (total cyanide) (μg HCN g^{-1} fresh root)

*: Values in bold and underlined are the five lowest for TCy and the five highest for other traits.

Genotypic values

The genotypic values (μ+g) of the 26 clones are shown in Table 5. The five best genotypic values (five lowest of TCy and five highest of the other traits) are highlighted in bold and underlined. Of the five best clones in terms of RY (clones 3, 8, 10, 16 and 23), three (10, 16 and 23) are among the top five in terms of DMC. Starch plays an important role in the cooking of cassava roots (Bechoff et al. 2018). Regarding TC, BC and TrBC, four clones (4, 5, 14 and 17) have the highest genotypic values in all, reflecting the high genetic correlations among them. Clone 4 has the best overall performance. Its genotypic values of DMC (33.37%), TC (10.51 μg g^{-1}), BC (8.77 μg g^{-1}), TrBC (7.85 μg g^{-1}) and TCy (38.64 μg HCN g^{-1}) are among the top five and, although the genotypic value of RY (15.19 t ha^{-1}) is not among the five highest, it is very close to the lowest value among the five highest (18.30 t ha^{-1}). This demonstrates that it is possible to obtain individuals with adequate means in all the important traits, in the context of cassava biofortification.

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